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THE DEVELOPMENT OF HYDRA.

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Introduction.

The development of hydra has been described by Kleinenberg (4), Kerschner (5), Brauer (3), and a few others, yet there are many points of interest and importance that have not been presented, especially in the origin and formation of the ovary, in fertilization and in early cleavage.

The following results are based mostly upon one species: *Hydra* sp.? (Brauer) or *H. diœcia* (Downing). The three forms *H. viridis, H. grisea and H. fusca* are universally recognized as distinct species. *Hydra* sp.? (Brauer) has been observed by many investigators, yet some prefer to consider it as a variety of *H. fusca.* Brauer at first placed it with *H. fusca,* but upon discovering that the sexes were separate, designated it as *Hydra* sp.?. Downing (2) in his paper on "Spermatogenesis of *Hydra*" uses the name *H. diœcia.*

Hydra sp.? (Brauer) which varies in color from a light to a dark brown, resembles *H. fusca.* When we take into consideration, however, that the sexes are separate, that the eggs are glued to the object on which the parent rests by a secretion from the ectoderm, and that the embryo may hatch out while the egg is still attached to the parent form, it is perhaps justifiable to regard it as a distinct species.

Origin and Formation of the Ovary.

The early formation of the ovary differs from Kleinenberg's account as it is usually given in text-books. The interstitial cells, which divide with nearly the same rate over the body of

the hydra, are about uniform in size, shape and appearance. The formation of the ovary first becomes recognizable by a rapid growth of the interstitial cells in some special region and not by their more rapid division. After these cells have increased several times in volume, they become differentiated into two distinct regions, namely, a more central region, which gives rise to the ovum or ova, and a peripheral region which may be considered the temporary ovary, whose cells cease to enlarge and later contribute directly to the formation of the yolk (Pl. VIII., Fig. 2). The ectodermal cells, with their scanty supply of cytoplasm and nuclei that stain very faintly, are pushed more to the exterior by the enlarged interstitial cells. They often remain connected with the mesoglea by fibrous strands.

The cells of the central region vary in number and their contents contribute directly to the formation of the ovum or ova (Fig. 2, *a*, *b*, *c*). Not merely one but all of these cells continue to enlarge. This increase in size affects the nuclei as well as the cell bodies. The cell walls break down and the cytoplasm which now becomes a common multinucleate mass without any definite outline comes to lie between the enlarged cells of the peripheral region of the ovary and the mesoglea (Fig. 3). The egg at this stage of development as stated above is multinucleate. All of the nuclei enlarge somewhat, the chromatin assumes the spireme condition and the nucleoli are very prominent. One of these nuclei, seldom more than one (Fig. 3, *a*), continues to enlarge and becomes the egg nucleus. The remaining nuclei gradually break down and disappear within the cytoplasm. When two nuclei persist, the cytoplasm becomes separated into two distinct parts. Each part with its contained nucleus becomes a separate egg, which develops independently of the other. The egg does not begin as a single cell, but as a multinucleate mass which results from the fusion of several cells after the breaking down of their walls. Thus, the cytoplasm which originates from several cells becomes the cytoplasm of the egg. Up to this stage of development it is very difficult to determine the exact origin of the sexual organs except from a study of sections. The ovaries and spermaries both begin by a rapid growth of the interstitial cells, but in the formation of the spermary, when the

interstitial cells have increased two or three times in volume, they begin to divide mitotically and give rise to the spermatogonia. In case of the ovary, on the other hand, there is no division of the interstitial cells after they have begun to enlarge.

As the egg nucleus enlarges, the cytoplasm sends out pseudopodial processes (Fig. 4), which form very rapidly and often encircle the entire body of the hydra.

According to Kleinenberg (*H. viridis*), in a zone which surrounds half of the body of the hydra there appear between the neuromuscle cells (ectoderm cells) small tongues of interstitial cells, the nuclei of which are so closely pressed together, that it is hard to distinguish between nucleus and cell body. He furthermore states that by a progressive multiplication of these cells the neuromuscle cells are pushed aside and the tongues of interstitial cells unite with each other, forming a single-layered oblong plate of cells between the ectoderm and endoderm. When the ovary has reached this stage of development, one of the cells, which is situated near the middle of the oblong plate, grows much faster than its neighbors and becomes the egg. The egg cell sends out pseudopodia, which grow very rapidly between the cells of ovary. After the pseudopodia have reached their maximum development they are drawn in, and the egg is completely formed. The cells surrounding the egg break down and act as food for it.

The tongues of interstitial cells which Kleinenberg speaks of are found not only in the region where the ovary begins, but in other parts of the hydra as well. They are especially abundant in hydra that are budding vigorously. The pseudopodia do not grow out between the cells of the ovary, but rather between the ovary as a whole and the mesoglea (Figs. 4 and 6, *ps*).

During the growth of the egg the cytoplasm becomes vacuolated. The nuclear membrane is very indistinct, but the nucleoplasm becomes very dense and granular (Fig. 4, *a*). Several of the degenerating nuclei are still visible in the cytoplasm. In Fig. 5, a stage a little later, they have entirely disappeared. When the egg has reached its growth, it is amœboid in form with the nucleus near the center. The egg at this stage of development contains no yolk (Figs. 4 and 5), but when the pseudopodia are completely formed, the nuclei of the interstitial cells forming the

ovary are taken up by the amœboid egg and become changed into the yolk or pseudo-cells of the egg. Fig. 6 represents a cross-section of several pseudopodia into which the nuclei of the interstitial cells of the ovary are passing. The transformation of these interstitial cells into yolk is shown in Fig. 7, *a–e*. The chromatin becomes very granular and forms a band around the inner border of the nuclear membrane. The nucleolus becomes imbedded in this band of granular chromatin and the nucleus has the appearance of a hollow sphere with its wall thickened on one side. After the yolk or pseudo-cells are formed they divide amitotically.

According to Kleinenberg, the interstitial cells of the ovary surrounding the egg break down and act as food for the developing egg. Brauer says the interstitial cells, after breaking down, enter the egg and give rise to the pseudo-cells.

After the amœboid egg becomes filled with yolk, the pseudopodia are drawn in and the egg becomes nearly spherical (Pl. XI., Fig. 33, *a*), and is surrounded by a single layer of ectodermal cells except at its base. The egg nucleus during the contraction of the pseudopodia becomes very faint and is difficult to recognize. According to Brauer, the nucleus becomes entirely invisible.

Abortive ova are often found in sexually reproducing hydra. They consist of a small mass of yolk cells surrounded by a thin egg membrane, and are devoid of a nucleus. The ovary begins as in the normal cases, but instead of one of the nuclei persisting in the multinucleate cytoplasmic cell mass, they all break down, leaving the common mass of cytoplasm without a nucleus. No pseudopodia are formed. This condition would be represented in Pl. VIII., Fig. 3, if no nuclei were present in the cytoplasm between the enlarged interstitial cells and the mesoglea. Some of the nuclei of the interstitial cells enter the cytoplasm as in the normal egg and form the yolk. The common mass of cytoplasm with its contained yolk now becomes spherical. The abortive ova do not break through the ectoderm, but are gradually absorbed.

Maturation.

Immediately after the pseudopodia are drawn in, the polar bodies are formed (Pl. XI., Fig. 33, *a*, *pb*). When formed they

remain attached to the egg by means of a cytoplasmic thread and are found partly imbedded in the egg membrane beneath the ectoderm surrounding the egg. When maturation is completed an opening breaks through the ectoderm in the region of the polar bodies. The egg with its contained yolk is very plastic, and as the ectoderm contracts or is drawn back, the egg contents is gradually forced through the small opening in the ectoderm. It requires from one to three minutes for the contents of the egg to pass through the opening of the ectoderm. The different changes which the egg undergoes in this process are shown in Pl. X., Figs. 27–32. The egg now becomes situated in a basin-like cavity of the ectoderm and is entirely free except for a small portion at the vegetative pole (Figs. 31 and 32), where it is finally attached to the cup-shaped ectoderm by means of transparent pseudopodial processes of the egg membrane, which pass into the ectoderm. An adhesive substance which is secreted by the ectoderm also aids in their attachment. The egg membrane is very tough and firm, and remains distinct during cleavage.

The polar bodies after the egg passes to the exterior become free in the water (Pl. VIII., Fig. 1). They are more distinctly shown in Pl. IX., Fig. 8, *pb*, with their connecting thread of cytoplasm. The chromosomes of the polar bodies do not become a homogeneous mass but retain their individuality. The first polar body is larger than the second. The connecting thread of cytoplasm becomes finer and longer, until it breaks loose from the egg and leaves the polar bodies free in the water, where they almost immediately go to pieces. Their connection with the egg may persist until after the third cleavage, as shown in Pl. XI., Figs. 36, 42.

According to Brauer's account, the polar bodies disappear before cleavage begins. I was unable to distinguish any movement of granular substance through the connecting thread between the polar bodies and the egg, but, as the granular cytoplasm of the strand is similar to that of the egg, it is highly probable that such a flow of substance occurs.

Fertilization.

Normally fertilization occurs within two hours after the egg becomes free in the water. There is a small cavity formed at the

point where the sperm enters the egg (Brauer). The egg becomes surrounded by a number of sperm, several of which may pass into the egg membrane, but only one enters the egg. It is interesting to note that the egg may remain susceptible to the sperm twenty-four hours after it passes through the ectoderm, but if the sperm are not added within twenty-four to thirty hours after maturation or the passing of the egg to the exterior, fertilization will not take place.

Two lots of hydras with eggs, one twenty-four hours and the other immediately after maturation, were placed in separate vessels containing water and sperm added. Fertilization occurred in both instances within two hours. The rate of cleavage was similar in both cases. In the unfertilized eggs, the yolk spheres cease dividing, gradually break down and the egg becomes a hollow membranous sphere containing a fluid substance.

Almost immediately after fertilization the peripheral cytoplasm becomes free from yolk and is more finely granular than the cytoplasm within the egg.

The entrance of the sperm and union of male and female pronuclei agree with Brauer's account. After the union of the pronuclei the cleavage nucleus passes a short distance into the egg from the animal pole and divides (Pl. IX., Fig. 9).

Cleavage.

The cleavage of *Hydra* sp.? is total, unequal and regular. Brauer states that the cleavage is equal and total, but he gives no figures to show the early cleavage stages. Kleinenberg says the cleavage (*H. viridis*) takes place in a remarkable manner and that pseudopodia or cleavage papillæ are formed at the point where the first cleavage begins. He also describes the second cleavage as very erratic, and states that the egg undergoes peculiar changes during cleavage.

The cleavage of *Hydra* sp. ? does not exhibit such erratic conditions as Kleinenberg describes for *H. viridis*.

Just before the first cleavage the egg changes from a spherical to a more oblong form, and the cleavage passes through the short axis. This peculiarity is true only of the first, second and third cleavages, being especially well marked in the first and second.

The first cleavage begins at the animal pole. The eggs of *Hydra* sp.? do not show any blunt projections or cleavage papillæ, as Kleinenberg and Andrews describe in *Hydra viridis*. As the first cleavage furrow deepens, pseudopodia are formed, which project into the cleavage furrow (Pl. XI., Fig. 34). These pseudopodial projections change in shape by contraction or expansion as the cleavage furrow progresses from one side to the other. The pseudopodia of one side may fuse with those of the opposite. When this occurs, they do not pull apart but remain connected during cleavage. Those that do not fuse are soon drawn in. The living material shows a movement of the granular cytoplasm containing a few yolk spheres from one blastomere to another through these connections. New pseudopodia continue to form until the cleavage furrow reaches the opposite side. When the cleavage is nearly complete the furrow closes at the point where it first began (Fig. 35), and when completely closed the pseudopodial connections are no longer visible in the living egg. The bottom of the cleavage furrow shows a distinct opening which extends entirely through the egg laterally and progresses with the cleavage from the animal to the vegetative pole (Fig. 35). This interesting phenomenon was observed by Kleinenberg and Andrews in *H. viridis*, especially during the first cleavage. Fig. 36 represents the first cleavage completed and the relation of the egg to the ectoderm as it appears in the living hydra. Sections of the different stages of the first cleavage are shown in Pl. IX., Figs. 9–11. After the first cleavage is completed, the nuclei divide before the second cleavage begins (Fig. 11). Brauer states that the second cleavage begins before the first is completed.

The second cleavage passes through a plane at right angles to the first and is nearly equal. This cleavage is similar to the first, as shown in Pl. XI., Figs. 37 and 38. A polar view of the second cleavage is shown in Fig. 39. A section of the four-cell stage taken at right angles to the polar axis (Pl. IX., Fig. 12) shows a number of pseudopodial connections or bridges persisting. The third cleavage differs from the first and second in that no pseudopodia are formed in the cleavage furrow. Pl. XI., Figs. 40–42 represent the different stages of the third cleavage as it appears

from side view in the living material. The third cleavage furrow which passes entirely through the egg instead of becoming obliterated within, as in the first and second cleavages, becomes the cleavage cavity. Pl. IX., Fig. 13 represents a section passing through the poles of the egg shortly after the third cleavage is complete. The cleavage cavity is very distinct and shows the nuclei near the inner ends of the cells. The egg now becomes more spherical and the inner ends of the cells become rounded off. The blastomeres of the vegetative pole are larger than those of the animal pole. The fourth and fifth cleavages are parallel to the third, but there is some irregularity in their time of formation. Both cleavages may begin at the same time, but in most instances observed the cells above the equator divided first. They differ from the first, second and third cleavages in that the cleavage does not start at one side and gradually pass to the opposite, but instead begins at different points on the surface at the same time. The cleavage furrows are very indistinct. Pl. X., Fig. 14 represents the fourth and fifth cleavages completed, as they appear in a plane passing through the poles. The cleavage cavity is very irregular and increases considerably in size (Pl. IX., Fig. 15) without any further division of the cleavage cells. This peculiarity is due to a change in the form of the cells, whereby their long axis becomes really tangential. The cleavage cells now divide very rapidly and it is impossible to distinguish any further regularity in the process of cleavage (Fig. 16).

Origin of Endoderm.

When the cleavage cavity reaches its maximum growth, the embryo consists of a large spherical blastula with its single layer of primitive ectodermal cells, which have about the same thickness throughout; but some of them now begin to enlarge, so that they come to project into the cleavage cavity (Pl. X., Fig. 17), and instead of dividing parallel to the surface begin to divide at right angles to it. The inner ends of the divided cells become free in the cleavage cavity and give rise to the endoderm. A section passing through the equatorial plane (Pl. IX., Fig. 18) shows the cells not only dividing radially but also tangentially. This process continues until the cleavage cavity becomes filled with cells

(Fig. 19). The cells within the cleavage cavity also divide very rapidly (Pl. X., Fig. 20), and the embryo becomes a solid spherical mass of cells with the cleavage cavity entirely obliterated.

The formation of the endoderm begins uniformily at the different poles of the blastula. Its origin is multipolar as Brauer states. According to Brauer, during the formation of the endoderm some of the cells which divide radially and remain within the wall of the blastula force the primitive ectodermal cells, which have a narrow base, from the periphery into the cleavage cavity before they divide, and thus an entire cell of the primitive ectoderm becomes an endodermal cell. The species studied showed a number of cells dividing radially in the periphery during the formation of the endoderm, but I was unable to find any indication that entire cells were forced from the periphery into the cleavage cavity.

According to Kerschner (5) and Korotneff (6), the endoderm is formed by the inwandering of cells from the vegetative pole of the egg.

When the endoderm is completely formed division ceases and the endodermal cells with their abundance of yolk can readily be distinguished from the cells of the outer layer or ectoderm.

Egg Membranes.

The outer and inner egg membranes are formed from the ectodermal cells. The outer membrane begins as an outgrowth from the different cells of the ectoderm. A very small portion or nearly all of an ectodermal cell may take part in this process (Pl. X., Figs. 20–22). These outgrowths in the early formation of the membrane remain continuous with the cell from which they originate, and are often nearly as large as the body of the cell itself (Fig. 23, *o*). The outer protoplasmic ends of these projections assume various shapes (Fig. 25). The thin elastic wall surrounding these outgrowths, which is a continuation of the wall of the ectodermal cell, often becomes very delicate, breaks through and allows the cytoplasm of the cell to flow out, thus causing the cell to collapse. The different outgrowths now fuse at their basal ends and form a continuous membrane around the developing embryo (Fig. 25). This membrane becomes very tough and has

the nature of chitin. When it is nearly formed, a second or inner membrane begins as a secretion from the ectodermal cells over the entire embryo just beneath the outer egg membrane (Figs. 25 and 26).

According to Kleinenberg, the entire primitive ectoderm is used in the formation of the outer and inner membranes. The formation of these membranes in *Hydra* sp.? confirms Brauer's account in that the inner ends of the ectodermal cells persist and become the definitive ectoderm.

After the membranes are formed, the eggs are glued to the object on which the parent rests. This, however, is not always true. In a few instances observed, the embryo hatched out while the eggs were yet attached to the parent. Brauer states that the eggs are glued by a sticky secretion from the ectoderm to the object on which the parent rests, and that the parent remains in contact with the egg until the embryo hatches out.

The formation of the interstitial cells and of the body cavity was not studied. The embryo hatches out in from eight to ten days after the outer and inner membranes are formed.

General Remarks.

The condition necessary for the appearance of sexual organs in hydra has long been a question of much interest, especially among scientific investigators. Various chemical solutions as well as different conditions of food and temperature have been tried with little or no success.

Downing (2) by subjecting hydra to various degrees of reduced temperature was able to get hydra to produce sexual organs after an exposure in a dark refrigerator to a temperature of about 12° C. for twelve hours. But as sexual organs appeared at the same time on several control hydras in the laboratory which were kept in the light at the temperature of the room, he concluded that light and temperature are not controlling factors in determining the appearance of sexual organs.

During the three different years that Hydra sp.? were collected at intervals from two to three times a week, no sexual organs were found, but buds were abundant in the winter season as well as during the warmer months. From this I was led to infer that

in this particular locality conditions were antagonistic to the development of sexual organs, but favorable for budding. After the hydras were brought into the laboratory and put in aquaria with abundance of food and the water kept well aërated, they reproduced by budding more rapidly than out of doors. As many as four and sometimes five buds were found on the same individual. After the hydras continued to bud very vigorously from two to six weeks, ovaries and spermaries were produced. Buds and ovaries or spermaries are often contemporaneous on the same individual. Sexual organs were never found on the buds. But if the buds after becoming mature were supplied with plenty of food, they in turn would produce sexual organs after passing through a stage of vigorous budding, as described above. The time for the appearance of the sexual organs on different individuals varied with the rate of budding.

According to Downing when buds and spermaries were found on the same individual, the spermaries appeared on the vigorous bud.

The hydra in most instances continued to feed during sexual reproduction. The endodermal cells at the time of the first formation of the sexual organs are gorged with food and protoplasmic granules, while the ectodermal cells are less granular and begin to show vacuoles. During the early formation of the sexual organs the endodermal cells, especially in the region of the sexual organs, become less granular and are almost free from food granules. This condition is most striking while the pseudopodia of the egg are forming. The pseudopodia grow so rapidly that the digestive process can not keep pace. But when the pseudopodia are completely drawn in, the endodermal cells immediately ingest food and show their former granular condition. But in those hydras that cease feeding during the sexual period, the endodermal cells remain non-granular and large vacuoles appear. The size of the egg in *Hydra* sp.? varies considerably with the amount of food present. This condition indicates a rapid use of the nutritive material during the development of the ovum.

Hydras that were kept in aquaria in a starved condition never produced sexual organs and very seldom budded. The ecto-

dermal and endodermal cells were similar to those in hydra during the formation of the sexual organs. This would indicate that lack of food and production of sexual organs give similar effects. Sexual organs never appeared on well-fed hydras, except those that had passed through a vigorous process of budding for a definite time. This indicates very forcibly that food is not a direct controlling factor in the production of sexual organs, but instead favors vigorous budding, which in turn gives rise to conditions that cause the appearance of these organs in the animal's life history. The question immediately arises, what are these conditions? Is it some inherent factor within the interstitial cell that has to do with the appearance of the sexual organs irrespective of food, temperature, etc.; or is it perhaps the inability of the cells in general to assimilate the food present after an active process of budding which leads to the differentiation of sexual organs in some definite region so that a new cycle may be started? The latter view seems the more plausible, as the ectodermal cells do pass through a marked change in those hydras that have been budding actively for some time. The cells become less granular, numerous vacuoles appear, the nuclei stain less intensely and very seldom divide. These conditions, however, vary in different parts of the same hydra. Moreover, as the ectodermal cells show this somewhat degenerate or exhausted condition in the parent hydra that is actively feeding and budding, it suggests very forcibly, as stated above, the inability of the ectodermal cells to assimilate the food present.

Summary.

The interstitial cells which give rise to the ovary, after increasing in volume become differentiated into two distinct regions: a central region which contributes directly to the formation and growth of the ovum, and a peripheral region whose nuclei later enter the egg and become changed into yolk.

Occasionally two nuclei persist in the central region and give rise to two distinct ova. Each ovum has its individual membrane and is entirely independent of the other. The ova are forced through the small opening of the ectoderm to the exterior by the contraction or pulling back of the ectoderm. The polar bodies

remain attached to the egg until after the third cleavage by means of a connecting thread of cytoplasm. When the eggs are not fertilized they disintegrate and finally fall to pieces.

The cleavage is total, unequal and regular. The cleavage cells communicate with each other by means of protoplasmic bridges or connections. There is a passage of substance from one cleavage cell to the other. The cleavage cavity begins with the third cleavage. The blastula when completely formed is a hollow sphere of primitive ectodermal cells.

The origin of the endoderm is multipolar. The outer and inner egg membranes are formed from the ectoderm; the first is formed by an outgrowth of the ectodermal cells, the second by means of a secretion from the same cells. The inner ends of the ectodermal cells persist and become the definitive ectoderm. The gastrula consists of a solid spherical mass of cells surrounded by the egg membranes. The eggs are either glued to the object on which the parent rests or remain attached to the parent until the embryo hatches. The eggs of *Hydra* sp.? will not continue to develop when removed from the parent after fertilization occurs, or even after cleavage has begun.

Hydras seldom continue to reproduce by budding after the sexual generation is completed. Exhaustion due to vigorous budding precedes the appearance of the sexual organs, more especially the ovaries.

Hydra sp.? reproduces by budding during the entire year. No sexual organs were found on the hydras when collected, but after the animals had been kept in aquaria with abundance of food, sexual organs appeared on those hydras that had been budding vigorously for several weeks. Spermaries or ovaries never appeared on buds.

It gives me pleasure to express my gratitude to Dr. George Lefevre for reading this manuscript.

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UNIVERSITY OF MISSOURI,
January 30, 1908.

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EXPLANATION OF PLATE VIII.

FIG. 1. Egg with polar bodies, immediately after it has passed to the exterior of the ectoderm. *c.m.*, egg membrane.

FIG. 2. Section showing early formation of ovary at the time the interstitial cells become differentiated into two distinct regions; *a*, *b*, and *c*, cells of the central region that are directly concerned in the formation of the ovum or ova; *p.r*, cells of the peripheral region which contribute to the formation of the yolk. × 76.

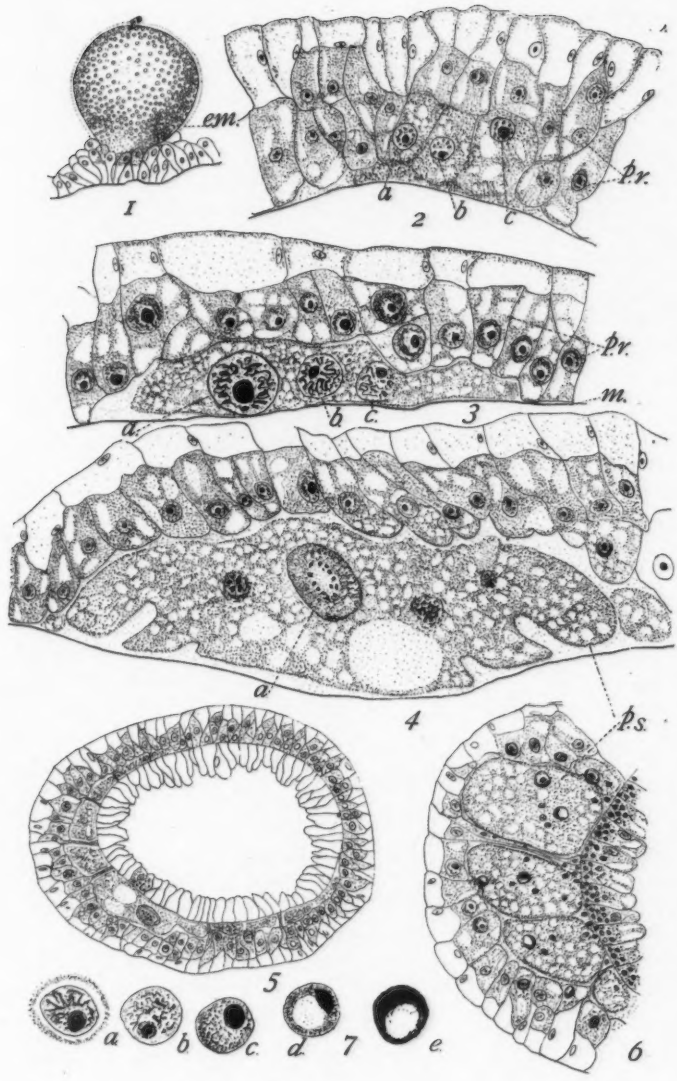
FIG. 3. Section, little later than preceding, showing the ovum between the interstitial cells, which later become the yolk, and the mesoglea; *a*, nucleus, which becomes the egg nucleus; *b.c*, nuclei breaking down within the egg cytoplasm; *m*, mesoglea.

FIG. 4. Section of ovum showing the formation of the pseudopodia; *a*, egg nucleus; *p.s*, pseudopodia. × 80.

FIG. 5. Cross-section of ovary, little later than preceding, just before the formation of the yolk or pseudo-cells.

FIG. 6. Cross-section of several pseudopodia showing the entrance of nuclei of the interstitial cells of ovary, which become the yolk.

FIG. 7. *a–e*, five stages in the transformation of interstitial nuclei into yolk.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Explanation of Plate IX.

Fig. 8. Section through the animal pole of egg showing polar bodies with connective thread of protoplasm; *p.b*, polar bodies; *pr.n*, pro-nucleus; *e.m*, egg membrane. × 750.

Figs. 9 and 10. Sections of eggs passing through poles, showing first cleavage and protoplasmic connections between cleavage cells: *p.c*, protoplasmic connections.

Fig. 11. First cleavage completed, and division of nuclei for second cleavage. × 55.

Fig. 12. Section of egg at right angles to polar axis, showing second cleavage complete. × 58.

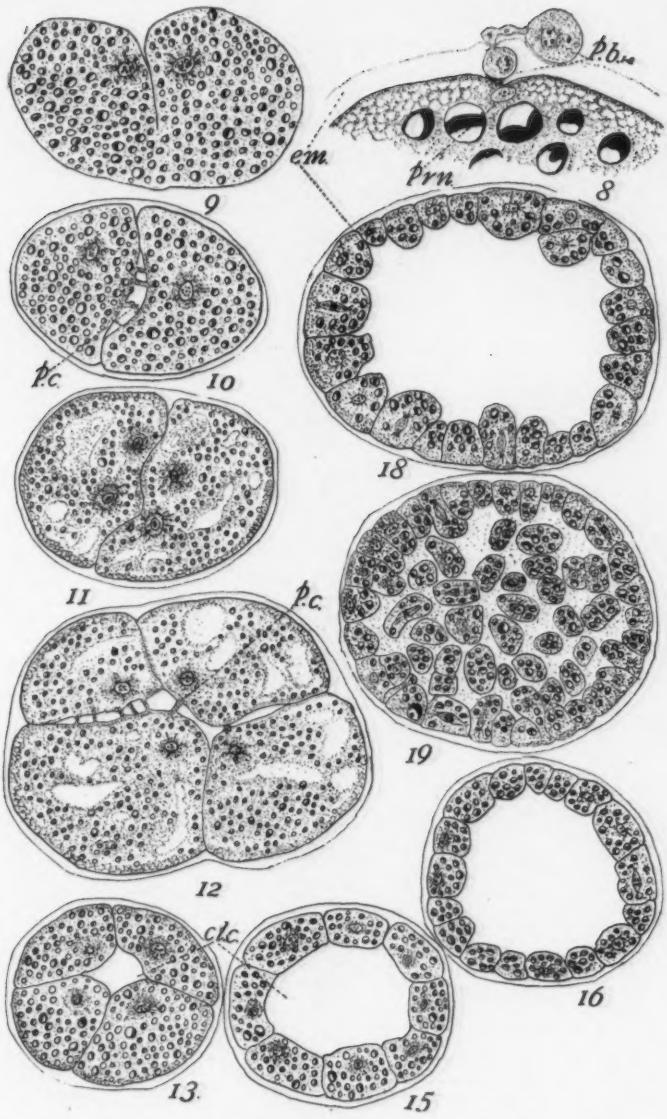
Fig. 13. Section of egg passing through poles with third cleavage completed and beginning of cleavage cavity; *cl.c*, cleavage cavity.

Fig. 15. Fourth and fifth cleavages complete. Cleavage cells becoming flattened out around the cleavage cavity.

Fig. 16. Stage a little later than preceding.

Fig. 18. Maximum development of cleavage cavity; primitive ectoderm cells dividing to form endoderm.

Fig. 19. Cleavage cavity becoming filled with endodermal cells.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Explanation of Plate X.

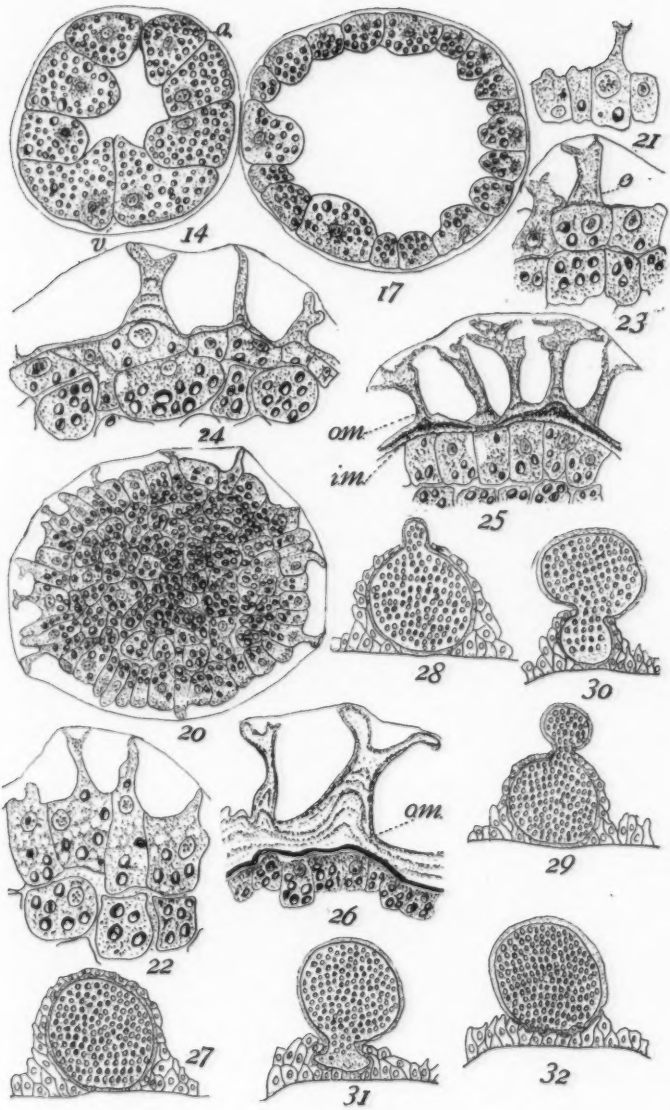
Fig. 14. Fourth and fifth cleavages completed; *a*, animal; *v*, vegetative pole.

Fig. 17. Blastula with primitive ectoderm cells, just before the formation of the endoderm.

Fig. 20. Cleavage cavity completely filled with endoderm cells and the beginning of the outer egg membrane.

Figs. 21–26. Formation of the outer and inner egg membranes; *o*, outgrowths.

Figs. 27–32. Different shapes which the egg assumes in passing to the exterior of the ectoderm.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Explanation of Plate XI.

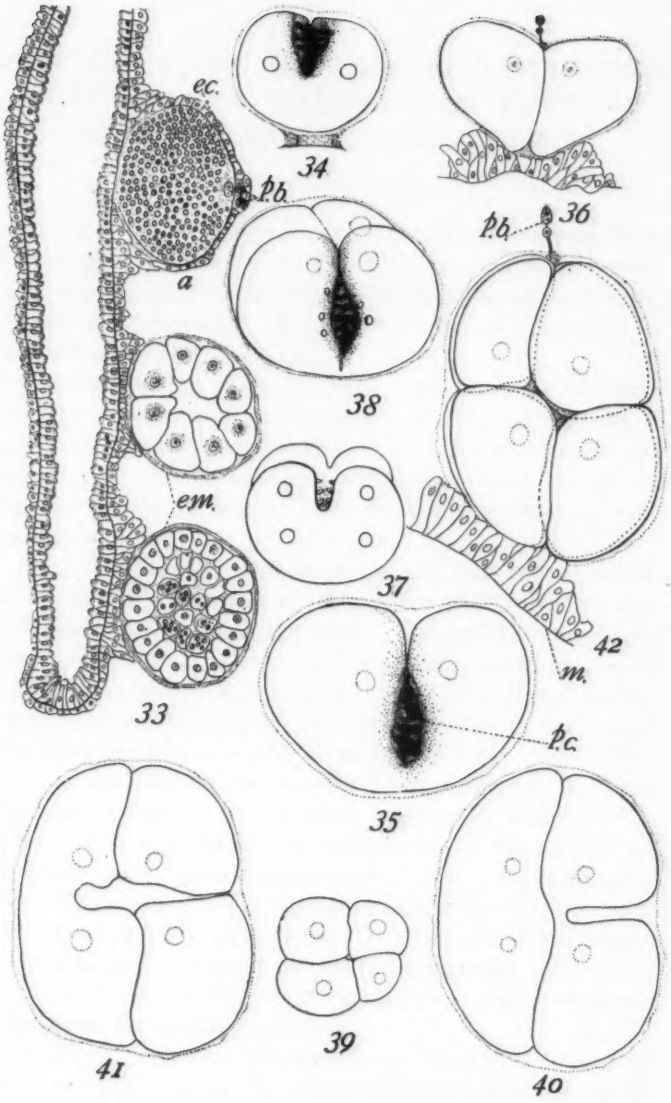
Fig. 33. Longitudinal section of aboral end of hydra, showing eggs in different stages of development; *e.c*, ectoderm surrounding egg; *p.b*, polar bodies; *prn*, pronucleus; *e.m*, egg membrane.

Figs. 34–36. Surface views of living eggs, showing first cleavage; side view; *p.c*, protoplasmic connections; *p.b*, polar bodies.

Figs. 37 and 38. Second cleavage, side view.

Fig. 39. Polar view of second cleavage.

Figs. 40–42. Third cleavage of living egg; side view; *p.b*, polar bodies.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SOME NOTES ON THE FACTORS CONTROLLING THE RATE OF REGENERATION IN TADPOLES OF RANA CLAMATA—DAUDIN.[1]

MAX MAPES ELLIS.

During the fall of 1907 some experiments were undertaken at the Zoölogical Laboratory of Indiana University at Bloomington, upon tadpoles of *Rana clamata* as a study of factors controlling the rate of regeneration. The results of this work are given in part in this preliminary report.

Tadpoles varying in body length from 35 to 40 mm. were arranged in series so that comparisons showing the influence of age, level of injury and location of injury (*i. e.*, whether the operation was in old or regenerating tissue) upon the rate of regeneration and the relation of first to second regeneration were made possible.

1. *Age.* — Tadpoles, 10 mm. whose tails had been removed. were used in this comparison. They were of four different ages with respect to the time of operation. The first set was operated upon on the date X, another on $X + 10$ days, a third on $X + 12$ days and the last on $X + 22$ days. Those injured on X had the highest rate of regeneration and those on $X + 22$ the lowest. The other two sets were both lower than the first. These differences in rate were not due to laboratory conditions as that point had been eliminated. As all other factors were controlled it is quite evident that the rate of regeneration of the tadpole tail decreases as the animal grows older. The averages are given here: Those of the date X regenerated at a rate of .39 of a millimeter per day; $X + 10$ days, .35; $X + 22$, .26. These figures show a decided decrease in the rate with an increase in age, 33⅓ per cent. of the rate being lost during the 22 days between the date of the first and last operations.

2. *First and Second Regeneration.* — The data collected are not at all conclusive for the relation of the rate of first regener-

[1] Contribution from Zoöl. Lab. of Indiana University, No. 94.

ation to that of second regeneration, age and level of injury being the same. Two comparisons were made and in the averages second regeneration is slightly less than first in one case and equal to it in the other. First regeneration .35, second .33; first .26, second .26. It is to be concluded from the averages that second regeneration differs very little from the first.

3. *Location of Injury.* — The effect of location was obtained from a set of tadpoles that had had 10 mm. of tail removed. After they had regenerated about 5 mm. they were again operated upon — one half 3 mm. caudad of first cut and the other half 3 mm. cephalad of the first cut. The first half gave a regeneration from tissue but recently laid down, the second a regeneration from old tissue in a tadpole that had just been regenerating from a level nearer the tip of the tail. Neither half varied greatly from the control but both were below it. The averages for those cut caudad of first injury are .23, control .24; .18, control .21; for those cut cephalad .41, control .46; .33, control .34. The difference here as in the comparison of first and second regeneration is in favor of the previously uninjured animal. It is not striking however, and shows that regeneration in either of the two cases presented by the two halves of this set, is almost the same as the first regeneration from the same level.

4. *Level of Injury.* — The rates of regeneration of tadpoles with 7, 10, and 13 mm. of tail removed were compared. From their simple rates it was quite evident that the rate of regeneration in the tail is directly influenced by the amount of tail removed. This is in direct accord with the work done by Spallanzani, who found the whole leg of a salamander to regenerate as soon as a part of it, and the more recent work of Morgan[1] on the tail of *Diemyctylus* in which he states, "the nearer the cut to the outer end the slower the rate of regeneration." However, in order to state this change in rate more concisely, the rate of regeneration was divided by the amount of tail removed and a "proportional rate" obtained. From this proportional rate it was found that the rate of regeneration varies not only directly but *proportionally with the distance the cut is removed from the tip of the tail.*

This point of proportional regeneration is now being worked up in detail in connection with the influence of age.

[1] *Jour. Ex. Zoöl.*, Vol. III., No. IV., 1906.

Two sets of tadpoles of different ages were used so the data given in the table below are divided into two parts.

Level of Cut in mm.	Rate of Reg.	Prop. Rate.
7	.24	.034
10	.34	.034
13	.43	.033
7	.19	.027
10	.26	.026
13	.34	.026

SUMMARY.

1. The rate of regeneration of a tadpole tail varies inversely as the age of the tadpole.

2. In successive regenerations from a given level the first and second regenerations are approximately equal.

3. Regeneration from recently regenerated tissue, and from old tissue of a tadpole tail that has been regenerating at a level nearer the tip of the tail, is almost the same as first regeneration from the same level.

4. The rate of regeneration in the tail of tadpoles is directly proportional to the distance of the level of the cut from the tip of the tail.

NOTES ON THE BREEDING HABITS OF AMBLYSTOMA PUNCTATUM.

ALBERT HAZEN WRIGHT.

Probably in early spring no amphibian eggs are more common in central New York than those of *Amblystoma punctatum*. And yet our knowledge of the salamanders themselves, from their first appearance at this season through the egg-laying period, is meager as compared with what is known subsequent to the deposition of their eggs. Of the early part of the breeding period I wish to record some notes made at Ithaca by various members of the Department of Neurology and Vertebrate Zoölogy during the last eight years.

Ithaca is located at the south end of Cayuga Lake valley. North and south of the city are large swampy areas. On the east, south and west are high steep hills, through which are cut numerous ravines. In these places and in marshy areas on the hills, *Amblystoma* is found to be very abundant in early spring.

At this season the salamanders migrate from winter quarters to suitable breeding-places, and the Ithaca marshes have always proved a favorite locality. Along the borders and through the middle of these swamps are several steam railroads and one electric railway. These prove an excellent check on the first appearance of *Amblystomas*, toads, wood-, meadow- and pickerel-frogs, and were it not for these railroads our records of first appearance would coincide with those of egg-laying.

In one place, where the electric railway passes near the mouth of a large ravine, 100–150 of *Amblystoma punctatum* are killed yearly. On April 6, 1906, 54 were counted, all having been killed the previous evening. The migration does not begin until dark. The street cars run until 11 P. M. and cross this spot about thirty times in an evening. When, as above stated, 54 were killed in four hours, what must be the number that cross these tracks during these intervals and after 11 P. M.!

The migration is quite clear. In the ravines in early spring we obtain the adults, but never their eggs. At the time the

salamanders cross the railway tracks, or a day or two subsequently, we record spermatophores and eggs in the ditches and swamp just beyond the track. Many individuals are slightly injured, and these we often find under cover near by. They almost invariably have bruised heads.

In equal abundance this form may be taken along the other borders of this swamp. From another region living individuals are secured in considerable numbers. As many as fifteen have been taken here within one hour, nor is it unusual to secure as many over the same area, two or three days later. During the day the salamanders crawl under the logs and loose railroad ties which lie along either side of the railroad embankments. A rake usually is employed, for it is under the ties partially submerged in the water that we obtain the largest number of individuals. At the southwest corner of Cayuga Lake they are found to be common under the leaves at the foot of the high perpendicular rock walls.

Three of our amphibia appear almost simultaneously: the spotted salamander (*Amblystoma punctatum*), the peeper (*Hyla pickeringii*), and the woodfrog (*Rana sylvatica*). If there is any difference or succession it is indicated by the order in which they are named. During the last eight years these species have appeared in spring as follows:

Amblystoma punctatum.	*Hyla pickeringii.*	*Rana sylvatica.*
1900, April 6	April 19	April 14
1901, April 13	April 12	April 13
1902, March 25	March 28	April 4
1903, March 13	March 15	March 19
1904, April 1	April 3	April 5
1905, April 1	March 29	April 2
1906, March 28	April 6	April 6
1907, March 24	March 25	March 28

From the above it appears that *Amblystoma* in six of these eight years preceded the peeper and the woodfrog.

The first record for 1902 was based upon twenty specimens taken by Professor H. D. Reed, from under leaves along the base of the perpendicular rocks of the west shore of the lake. "They were all found in groups of two each and proved, with one or two exceptions, to be male and female."

Whether a preliminary courtship similar to that recorded for the spotted newt (*Diemyctylus viridescens*) obtains with *Amblystomas* has always been problematic. Clarke[1] says his captive "males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they are kept." Smith[2] remarks that "in *Amblystoma* as in axolotl there is evidently no clasping of the female by the male such as occurs in *Triton* (*Diemyctylus*)."

Mr. A. A. Allen secured at Buffalo, N. Y., March 29, 1907, five individuals of *Amblystoma jeffersonianum*. Upon returning from his collecting trip he put them into a receptacle and immediately the smallest one (♂) embraced another (♀) exactly after the manner of the spotted newt. At Ithaca, April 2, after he had transferred them to a larger aquarium jar the same individual repeated the performance. The embrace was continued for sometime. Neither eggs nor spermatophores were subsequently laid, yet the fact is significant.

Of the intervals that exist between the first spermatophores deposited and the first eggs laid Professor Andrews[3] has noted that "24 hours or so" may intervene. Upon this point the following data may be of interest: in 1903, one interval of 2 days was recorded; in 1904, one of 4 days; in 1906, one of 6 days; in 1907, 4 days in one pond, 5 in another, and 7 in a third.

Egg-laying generally begins about the first of April. In two or three of the last eight years eggs have been noted before that date. In this period the earliest record is March 20, 1903. In 1901 they did not begin depositing eggs until after the middle of April. The egg-laying for the species may extend over a month or more. Rarely do we find fresh eggs after May 1. In 1907, our latest record for fresh eggs is April 30; our latest record for fresh spermatophores in the same pond, April 27.

[1] Clarke, S. F., "Development of *Amblystoma punctatum*, Studies from Biol. Lab. of Johns Hopkins Univ., No. II., 1880, p. 106.

[2] Smith, B. G., "The Breeding Habits of *Amblystoma punctatum* Linn.," *American Naturalist*, XLI., No. 486, June, 1907, p. 388.

[3] Andrews, E. A., "Breeding Habits of the Spotted Salamander (*Amblystoma punctatum*)," *American Naturalist*, Vol. 31, p. 636.

The first egg records for the last eight years follow:

1900, April	6	
1901, April	13	
1902, March	28,	3 days after first appearance of the species.
1903, March	20,	7 " " " " " " "
1904, April	5,	4 " " " " " " "
1905, April,	11	
1906, April	14,	17 " " " " " " "
1907, March	30,	6 " " " " " " "

So far as I am able to determine there are very few observations upon egg-laying in nature. In 1878, Samuel F. Clarke made the following observations upon some captive females: "I was interested to find, after carefully watching the process a number of times, that the number of eggs deposited at a time depends upon accident. If the creature is disturbed, as by another individual striking against or touching it, or by the moving or jarring of the dish, she immediately suspends operations, and seeks some more quiet spot for the continuance of her labors. I have seen a single egg deposited and again a bunch containing one hundred and fifty. While the eggs are being extruded the animal usually lies with its anterior limbs extended laterally, while the hind limbs are curved around the opening of the cloaca and appear to assist in holding together the eggs as they are laid."

Egg-laying apparently takes place almost entirely at night. A chance discovery made while studying the early breeding habits of our local *Anura* may tend to confirm this view. At 9 P. M. of March 30, 1907, I found the *Amblystomas* of one pond swimming restlessly about its edges in considerable numbers and suspected that egg-laying was about to begin. At 9:30 P. M. another pond was visited and by means of an electric flashlight three different females of *Amblystoma punctatum* were found in the egg-laying position.

This pond is 30 × 15 feet in diameter, 2–3 feet deep, and the banks steep. The bottom is covered with dead leaves. In the pond are brush and growing smartweed (*Polygonum Hydropiper*). About the pond the first adult *Amblystomas* were taken March 24; the first spermatophores were deposited in the pond March 25; and the first eggs laid March 30.

All three females were laying at one time, and two of the three were simultaneously depositing upon two closely apposed stems of smartweed, the vent of the lower one being about an inch below that of the other. One female held on with both hind- and fore-limbs. Her head was appressed to the side of the stem. The second female was facing the first, her vent being slightly lower. She grasped the stem only by her hind limbs, was semi-erect and inclined diagonally to the side. The stems were so small and close together, that had the second desired it she could not have clasped the stem with her fore-limbs without embracing the first female. In both the tail extended diagonally downward, no prehensile tendencies being noted in any of the three.

The first female after about a minute disengaged herself and swam off. The second after a short time, did the same but was captured. Both bunches of eggs were at this moment no more than one half of an inch in diameter. In less than an hour they were two inches in diameter. The third female, only a foot away had not been disturbed by the sweeping of the net. She held on by her hind limbs only, leaving most of her body free. The bunch of eggs was only an inch from the top of the stem. This female was not perfectly erect but slightly arched.

When the second female was killed she emitted many eggs. Evidently, the first bunch, normal in number, was not all she had to lay. Nor is it unusual to record *Amblystoma* eggs laid in small bunches. In one of our temporary woodland pools, March 30, 1907, I found a stem of common nightshade (*Solanum Dulcamara*) which had within a length of one and a half feet 14 bunches of eggs, 15–20 eggs to a bunch. It is very doubtful if each bunch represents a different female, when it is well known that a female may have 150 or more eggs to deposit. It is more natural to conclude that these bunches represent the egg complement of one. The oviposition might have been interrupted, the female might have crept along the stem or after a period of emission she might have risen to the surface for air and then returned to the stem again as the swamp cricket frog (*Chorophilus triseriatus*) regularly does.

In captivity females when depositing may have quite long

intervals between the emissions of eggs. This spring a female taken at the height of the breeding season voided enough eggs to cover the bottom of a small aquarium jar. She was brought into the laboratory without an attendant male and after several hours began to deposit eggs. The deposition lasted ten days and the eggs first laid hatched. Captive females have more of a tendency to lay single eggs than in nature, yet single eggs have been recorded afield. Even before the female reaches the marshes or ponds she occasionally lays eggs. In several instances near the breeding grounds we have found under moist stones or logs one or two eggs laid by *Amblystomas*.

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A NOTE ON THE CHEMOTAXIS OF OXYTRICHA ÆRUGINOSA.

GEORGE WAGNER.

Oxytricha æruginosa, Wrzeniowski, is one of the *Hypotricha* occurring rather irregularly in laboratory cultures. Cultures of it, so far as my experience goes, are quite characteristic in the fact that they are distinctly brown in color, and decidedly more alkaline than the average cultures of *Paramecium*.

Jennings and Moore[1] found that this organism differed from the congeneric *Oxytricha fallax* by forming spontaneously dense aggregations like those of *Paramecium*. On the other hand they found that unlike *Paramecium* it was not positive to carbon dioxide or indeed to any acid. They found that if both forms were mounted on the same slide according to Jennings's now well-known method, they would form separate aggregations, and that the members of each species would pass freely through collections of the other without being in any way detained. It is obvious therefore that the cause of aggregation in the two forms must be different.

Several years ago I made an attempt, at the suggestion of Professor Jennings, to learn somewhat more about this difference. Unfortunately I was unable at that time to complete the work, and I have not since been able to secure the necessary cultures of *Oxytricha*.[2] It seems wise therefore to publish the results so far obtained even though they may seem fragmentary.

The spontaneous aggregations of *Oxytricha* on slides or even in small open dishes are remarkable in being much more dense than those of *Paramecium*, in being much more sharply circumscribed, and in expanding much less rapidly. In certain mixed mounts of *Paramecium* and *Oxytricha* I found that, probably by mere coincidence, both forms congregated in the same area. In such cases at first the area inhabited by both was the same, ex-

[1] *American Journal of Physiology*, Vol. 6, 1902, pp. 242–243.

[2] For the rest of this paper *Oxytricha* is to be considered as meaning *O. æruginosa*.

cept that *Paramecium* swam further out, possibly due to the greater momentum caused by their larger body and faster rate of motion. But soon *Oxytricha* began to shun the center of the drop, repelled by the carbon dioxide exuded by the *Paramecia*. But they were held by their own attractive excretion in a ring around the outside of the area. Within thirty minutes however the *Oxytricha* had moved so far outward that the *Paramecia* were entirely separated from them; shortly afterward the aggregation of *Oxytricha*, due undoubtedly to its attenuated character, broke up, while the *Paramecia* were still densely crowded together. In a second similar group, where however the relative number of *Paramecia* was much smaller, the two organisms remained evenly distributed throughout the drop, and the area was extended at the same rate for both of them. Why this difference? Very likely because the smaller number of *Paramecia* acted less rapidly in making the center of the drop uninhabitable for *Oxytricha*, while the *Oxytricha* in moving outward, by their own excretion of carbon dioxide kept the whole area acceptable to *Paramecium*.

Paramecia in culture water, after aëration, react positively to distilled water or weak acid. When in distilled water they react positively to weak acid but negatively to stronger acid or to alkali. *Paramecium* therefore usually seems to seek an optimum region, weakly acid in reaction. (Of certain exceptions to this I hope to speak in another paper.) The relation of this to the general life activities of *Paramecium* has often been pointed out by Jennings.

A culture of *Oxytricha*, aërated in similar fashion, reacts quite differently. If such an aërated culture is placed under a cover glass on a slide, and a drop of distilled water is introduced, the center of this drop remains free from *Oxytricha*. Such as swim into the edge of the drop give the typical motor reaction, long before they reach the center, and then swim outward, but only for a short distance; for there exists now also an outer boundary bringing forth the same reaction. The result is that there is soon formed a ring-like aggregation, quite like that of *Paramecium* in presence of a drop of stronger acid. If such an aggregation is left undisturbed for some time, the *Oxytricha* usually soon begin to collect in some one portion of the ring, and within five or six minutes form there a very dense aggregation.

When similarly aërated cultures are tested with alkaline solutions it is found that with very weak alkalies, such as $n/1{,}000$ — $n/1{,}500$ KOH, the results were quite parallel with those with distilled water. That is, there was a ring-like aggregation around the edge of the drop. When the culture was made slightly acid, and then a drop of hydrant water (alkaline) was introduced, there was usually a more or less distinct ring formation, occasionally a complete negative reaction. The hydrant water used was decidedly more alkaline than the ordinary cultures of *Oxytricha*. That *Oxytricha* forms a ring around such a drop, might make one suppose that here we find an optimum of slight alkalinity for *Oxytricha*. But such proves not to be the case, at least not in so simple a form. Many experiments showed that so far as my cultures went, *Oxytricha* was always negative to stronger KOH ($n/200$) often with formation of a ring, and without exception wholly indifferent to weaker KOH. There was never a gathering within the drop no matter what dilution was used.

It seems then that there is really no optimum strength of alkali in which *Oxytricha* will gather. In those cases, just mentioned, where there is a ring formed, we must rather suppose that the alkali plays no part except to repel, but that the water acts as diluent to some other substance present in the culture, attractive to *Oxytricha*, but present in super-optimum amount.

In cultures made slightly acid, *Oxytricha* reacts to weak alkali either by collecting in it (rarely), or by forming a ring around it, depending on the strength of the alkali. Here again it is probably not an optimum condition somewhere in the alkali that causes the result, but rather a reduction to an optimum amount of some other substance present in the culture medium, coupled with a strongly negative reaction toward the acid. For if instead of a weak alkali we use a drop of culture liquid, which is normally weakly alkaline, we find an immediate and strong positive reaction in all cases. It is then not alkalinity but an unknown factor that produces this result. That alkalinity is not the factor is shown by further experiment.

In one culture it was found that *Oxytricha* when exposed in culture water to a drop of distilled water reacted purely negatively, and this whether the culture was aërated or not, or

whether the distilled water was aërated or not. Many repetitions always gave the same result. It seemed possible that here was a culture with optimum alkalinity. If so an increase in its alkalinity ought to produce ring formation around a drop of distilled water. To test this the culture was made rather strongly alkaline by addition of $n/100$ KOH, and to a mount a drop of distilled water was added. But the result was as before. In every case this one race, even when the culture was made many times more alkaline than in its natural state there was still a purely negative reaction toward distilled water. There was evidently an optimum condition in this culture, but it was not an optimum of alkali.

Some other cultures varied from this by showing only an incipient tendency toward ring formation with distilled water, in others again *Oxytricha* soon approached very close to the center of the drop. It seems obvious therefore that various cultures differ widely as to their approach to an optimum state, but that this variation is not, primarily at least, correlated with alkalinity. It seems plain to me, that the reactions of cultures to distilled water are due not to alkalinity but to the effect of some other yet unknown condition. Distilled water represents to *Oxytricha* not merely a transition stage between weak alkali and weak acid. If it did one might expect *Oxytricha* (always, when in culture solution, negative to even very weak acid) when placed in weak acid, to react positively to distilled water. A culture was made acid and aërated, then a drop of distilled water introduced on a mount. In some cases there was indifference, in a few there *was* a positive reaction, but in by far the greatest number of cases the organisms gave a negative reaction toward the distilled water, as most alkaline cultures would have done, sometimes with ring formation.

The same acidified culture however reacted positively to a drop of its own unacidified culture water.

It seemed obviously desirable to find out something of this unknown constituent leading to these results. Most of the experiments seemed to show that such a substance was present usually in more than optimum amount, hence the frequent occurrence of ring formations around diluents. It should be noticed

that in spite of our knowledge of the lack of reaction of *Oxytricha* to carbon dioxide, I took the precaution of aërating the cultures in all the above experiments, except where otherwise stated. The reason for this precaution lies in the following results.

A portion of an *Oxytricha* culture was taken and thoroughly aërated by squirting it repeatedly into a watch glass from a pipette. A mount was immediately made and a drop of non-aërated liquid from the same culture introduced. The result was a strong positive reaction toward this non-aërated drop. To an entirely similar mount a drop of *aërated* culture water was added. The organisms were entirely indifferent, swimming in and out of the drop, with no sign of reaction. Thirdly, a non-aërated mount was now made, and a drop of aërated culture water was added. The drop remained free from *Oxytricha*, which reacted negatively at its outer edge. If to a non-aërated mount a non-aërated drop was added, there was again indifference. These experiments were repeated many times, always with the same results. It is evident therefore that a non-aërated culture contains some substance toward which *Oxytricha* reacts positively, and that this substance can be removed by aëration. It must therefore be either volatile, gaseous or easily oxidized.

But an *aërated* culture will also react negatively to hydrant water or to distilled water, often with ring formation. That such ring formation occurs with distilled water shows that what happens here is due to another, non-volatile substance present in the culture, toward which *Oxytricha* also reacts positively at proper concentration. This second substance also seems to be present usually in super-optimum amount. This second substance may sometimes be the alkali. That it is not always such is of course well shown by the case mentioned above, where a culture, after addition of much KOH and of aëration, still remained purely negative to distilled water. This experiment also shows that it is not merely a matter of osmotic pressure.

An interesting experiment in this connection is that in which *Oxytricha*, previously gathered into hydrant water by electrotaxis, were mounted and a drop of slightly acidulated culture water added. There was quickly formed a dense ring around the drop, which was long maintained. It was evident that here

one or both the unknown substances attracted the organism, while the acid above a certain strength repelled it. The *Oxytricha* under the influence of these opposite forces, was held along a line where the two balanced each other. According to Jennings, an exactly similar phenomenon occurs in *Paramecium*, when exposed to a mixed solution of acid and table salt.

It appears then that we have here at least two unknown substances, one volatile, the other not, affecting the chemotactic reactions of *Oxytricha*. The presence of two such substances, whatever may be their origin, helps to explain away a difficulty which I think has been overlooked. In the ring formation so commonly occurring, the *Oxytricha* are at first distributed equally around the circle. They are held within it, so we suppose, because some substance is there in optimum quantity. As the drop, say of distilled water, diffuses this optimum area moves outward, and the organisms move with it. However, if undisturbed, the *Oxytricha* will soon be congregating in some one portion of the circle and forming a dense mass, entirely similar to the ordinary spontaneous aggregations. Now on the supposition that there is only one attractive substance concerned, such aggregation in one part of a circle which is supposed to uniformly contain an optimum amount of this attractive substance is hard to understand. But if we suppose that in the dilution of one of two attractive substances to an optimum strength the second substance is reduced below its optimum, then it is easily seen that the meeting of two or three *Oxytricha* in some part of the circle would soon tend to bring this second substance to its optimum strength in that part of the circle. Then naturally any further individuals entering this smaller area would be retained there, in similar manner to that by which they are retained in the circle as a whole.

I can only add that there is some evidence that these substances are formed with the development of a colony, and that the character of the chemotactic reaction itself is altered in the course of such development. A culture of *Oxytricha* rejuvenated by addition of fresh hay and water, acted very anomalously. The creatures were positive to distilled water, as well as to $n/100$ HCl. But two days later all this had changed, and the reactions to both substances were negative.

In older colonies the attractive substances seem to develop far above the optimum amount. In such culture water the amount can be again reduced by filtering and allowing the filtrate to stand over night in an open dish. Whether such reduction is by volatilization or oxidation, or by both, I do not know. Certain it is that to such a liquid *Oxytricha* taken from the same culture from which it came, react positively.

Ordinarily a mount from any culture will be indifferent toward its own filtered culture water, if both remain unaërated. But if from a culture a highly concentrated mass of individuals are taken and kept for a few hours in a small watch glass, and then mounted, they will give a strong positive reaction toward a drop of water from their own original culture. Evidently here a close aggregation of individuals has supercharged the water, hence a positive reaction to the original culture solution of lesser strength.

Peculiarly, of two colonies of *Oxytricha* each, unaërated, was negative to the culture water of the other, but when aërated it became positive, the introduced drop in both cases being unaërated. This again points to a marked difference between cultures, as several other facts do. To this phase of the subject I expect to recur in another paper.

In a search for a clue to the unknown attractive substances, I tried many organic acids, sugar, urea, acetic aldehyd, potassium cyanide, ammonia, and a few others, but always with purely negative reactions. The possibilities of the case are of course nearly endless, but should opportunity offer, I would wish to experiment with organic sulphur compounds, and with compounds of ammonia, and this purely on the empiric basis of the odors of *Oxytricha* cultures.

Zoölogical Laboratory,
University of Wisconsin,
January 27, 1908.

ON THE SEXUAL DIFFERENCES OF THE CHROMOSOME GROUPS IN GALGULUS OCULATUS.[1]

FERNANDUS PAYNE.

An examination of the spermatogenesis of *Galgulus oculatus* together with a comparison of the male and female chromosome groups, has brought to light a new type of sexual difference in respect to the chromosomes. With this is correlated a new type of distribution of the chromosomes in the formation of the spermatozoa. On account of the interest of these facts, both in themselves and in relation to the theory of sex determination advocated by McClung, Stevens and Wilson, it seems desirable at the present time to offer this preliminary note on the subject. As soon as additional material can be procured, I hope to study and describe the facts more in detail; but those here presented, are shown beyond a reasonable doubt by the material now available.[2]

The number of chromosomes in the first maturation divisions is somewhat difficult to determine with certainty, owing to the fact that associated with them are always a greater or less number of deeply staining bodies resembling yolk granules, large numbers of which are also present in the general protoplasm of the spermatocytes in this genus (Fig. 1, *F*, *G* and *H*). I hope to overcome this difficulty in later work by means of differential stains. These granules can, however, be distinguished from the chromosomes by their smaller size and spherical form. A comparison of a number of equatorial plates of this division shows that the number of chromosomes is almost certainly twenty. All of these divide equally so that the secondary spermatocytes receive twenty chromosomes each. In the specimens figured the chromosomes do not show any definite arrangement, but in some cases, fifteen

[1] By some later writers, the generic name of this species is given as *Gelastocoris*. For the sake of avoiding confusion, I shall retain the term *Galgulus*.

[2] This material was collected by Prof. E. B. Wilson, in North Carolina, and given me for study. I wish to thank him for helpful suggestions and criticisms. He also kindly made the photographs for me.

of them arrange themselves in the form of a ring, inside of which are the other five, which in this division are quite separate.

The chromosomes seem to vary considerably in size and shape, but this is merely due I think to various degrees of foreshortening, produced by slight differences of position. However, some are

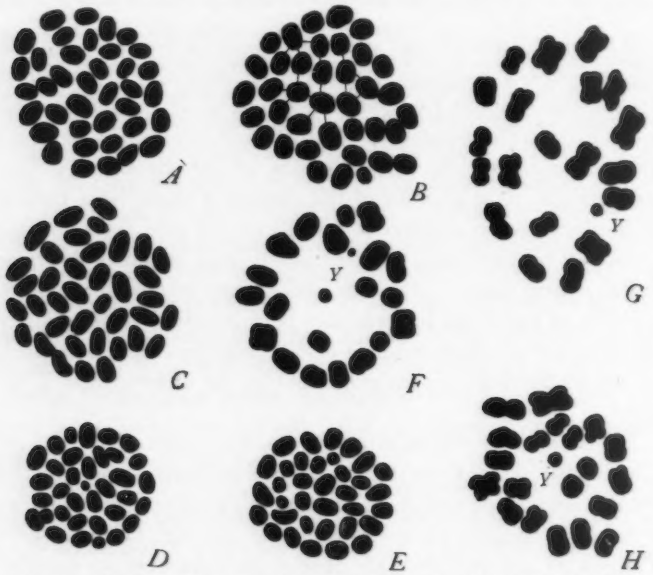

FIG. 1.[1] *Galgulus oculatus*. *A*, *B* and *C*, metaphase of female (oögonia or follicle) cells, polar view, showing thirty-eight chromosomes; *D* and *E*, metaphase of spermatogonial cells, polar view, showing thirty-five chromosomes; *F*, *G* and *H*, metaphase of the first spermatocyte division, polar view, showing twenty chromosomes and the granules γ. *A*, *B*, *C*, *D* and *E* are magnified 3,105 diameters and *F*, *G* and *H* 2,009 diameters.

evidently quadripartite and some bipartite. From my figures some may be inclined to believe that the granules may be chromosomes, but the number twenty is verified by the fact that the

[1] All of the figures were very carefully drawn with a camera, a 2 mm. oil immersion (Spencer) and compensation ocular 12 (Zeiss). Some were enlarged once and some twice by means of a drawing camera which magnified $1\frac{6}{11}$ diameters. Although some error is unavoidable at such an enlargement, I have used great care in correcting, and the chromosomes are not schematized in the least, as can be seen by a comparison of the drawings, Fig. 2, *B* and *F* with the photographs, Fig. 3, *A* and *D*.

metaphase figures of the second division, without exception show twenty chromosomes. Again I have counted the chromosomes. in serial sections of the prophases before the nuclear membrane breaks down. At this time, there are no granules within the nucleus and several counts showed twenty chromosomes each.

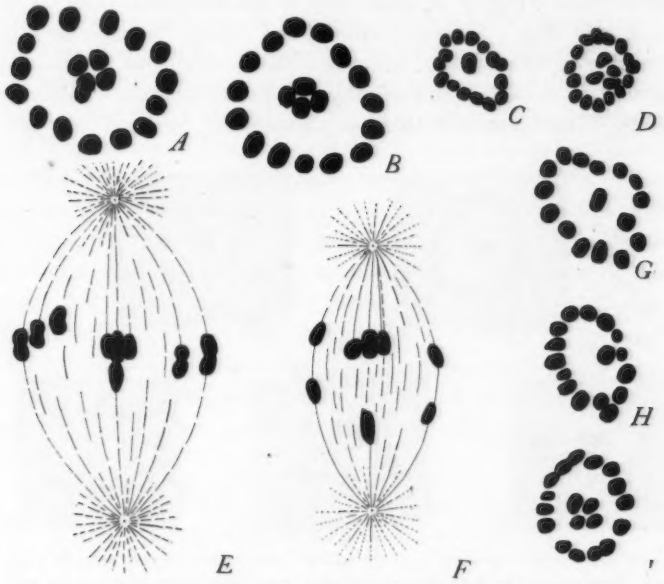

FIG. 2. *Galgulus oculatus.* *A* and *B*, metaphase figures of the second spermatocyte division, polar view, showing the ring of fifteen chromosomes and the pentad group in the center — in *B*, the chromosome beneath the four group could not be shown without displacing it; *C* and *D*, late anaphases of the second division, polar view, showing the unequal distribution of the chromosomes; *E*, side view of metaphase, second division, showing the typical arrangement and position of the pentad group — the spindle in both *E* and *F* is diagrammatic and merely shows size relations; *F*, side view of the early anaphase, second division, showing the manner in which the chromosomes of the pentad group separate; *G*, *H* and *I*, early anaphases of the second division, showing the chromosome distribution to the two classes of spermatozoa. All figures are drawn on the same scale and magnified 2,009 diameters.

The second division, which follows immediately after the first, shows a remarkable regrouping of certain of the chromosomes. Fifteen of the twenty take up the position of a ring, within which is a definite compound element formed by the remaining five.

These are now arranged in a pentad group, which always shows the same composition and occupies the same position. Four of these five chromosomes are grouped very closely together and lie in one plane, while the other one is either above or below this group of four, lying close to them on the other side of the equatorial plane (Fig. 2, *A*, polar view of the equatorial plate; Fig. 2, *E*, and Fig. 3, *C*, side views). The fifteen chromosomes in the ring divide equally, while the chromosomes of the central pentad do not divide individually, but the group as a whole separates in such a manner that one chromosome passes to one pole

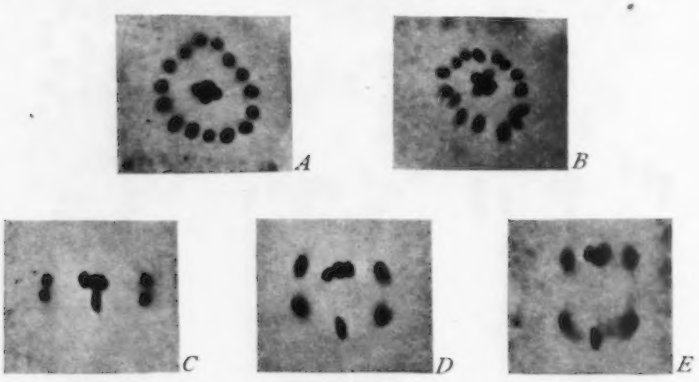

FIG. 3. *Galgulus oculatus*. *A*, metaphase figure of the second division, polar view, showing the ring of fifteen and the four chromosomes of the pentad group near the middle — the fifth chromosome of the pentad group could not be shown, as it lies beneath the four; *B*, early anaphase of the second division, polar view, showing the nineteen chromosomes which go to one pole; *C*, metaphase of the second division, side view, showing the typical position and arrangement of the chromosomes of the pentad group; *D* and *E*, anaphases of the second division, side view, showing the manner of separation of the pentad group, four chromosomes of which go to one pole and one to the other — only three chromosomes of the four group show, as all of them do not lie in the same plane. The photographic enlargement is 1,500 diameters.

and the other four to the other pole (Fig. 2, *F*, and Fig. 3, *D* and *E*). Two classes of spermatozoa are thus formed, which contain sixteen and nineteen chromosomes respectively. The early anaphase illustrating these two classes is shown in Fig. 2, *G*, *H* and *I*, and Fig. 3, *B*; the later anaphase in Fig. 2, *C* and *D*.

It yet remains to bring these two classes of spermatozoa into relation with the spermatogonial and oögonial numbers. Unfortunately but two spermatogonial metaphase figures are shown with entire clearness. They agree in showing each thirty-five chromosomes (Fig. 1, *D* and *E*). It may be thought that this evidence is not sufficient to establish the number with certainty. However, the number in these two cases is quite unmistakable, and as will be shown, it is the number to be expected from the numerical relations observed in the spermatocyte chromosomes and in the female. The female number (oögonia or follicle cells) is, without a doubt, thirty-eight (Fig. 1, *A*, *B* and *C*). The analogy which exists between the numerical relations here and in those forms with an odd chromosome, where the female number is one more than the male, makes the evidence all the more convincing that thirty-five is the male number in *Galgulus*. It is evident from these facts that the reduced female group must contain nineteen chromosomes ; and that accordingly females are produced upon fertilization by the nineteen-chromosome class of spermatozoa ; males upon fertilization by the sixteen-chromosome class.

Egg 19 + spermatozoön 16 = 35 (♂)
Egg 19 + spermatozoön 19 = 38 (♀)

So far, I have not ventured a new name for these characteristic chromosomes which make up the pentad group of the second division. At present I shall simply refer to them as differential chromosomes as Wilson, '06, has done in the case of the idiochromosomes.

Between the spermatogonial and the first spermatocyte divisions occurs a very prolonged growth period, during which the cell diameter increases approximately five times. Throughout this growth period, between synapsis and the formation of the chromosomes preparatory to the first maturation division, persists a large deeply staining body, more or less comparable in time of appearance and behavior, to the chromosome nucleolus of those forms in which it represents the odd chromosome or the idiochromosomes. I have not fully followed the history of this

structure in *Galgulus* and hence cannot definitely say what it is. It is possible that it is formed by the fusion of the five chromosomes which later appear in the pentad group of the second division.

At present there are not sufficient data to show the exact relation between this type of chromosome distribution and those already described. For this reason, I shall not attempt to homologize them in detail. Nevertheless, there is an evident similarity between the behavior of the pentad group as a whole and that of a single pair of idiochromosomes. In a general way, the four chromosomes of this group which go to one pole have the same relation to sex-production as a single large idiochromosome, while the one chromosome which goes to the other pole may be compared to a small idiochromosome.

With the discovery of this new type of differential chromosomes, it becomes more evident that the sexual differences of the chromosomes, even in the same order of animals, by no means conforms to a single numerical rule. In forms with one pair of idiochromosomes, the two sexes have the same number of chromosomes; in those with the odd chromosome, the female has one more than the male; and in *Galgulus*, the female has three more chromosomes than the male.

In many forms, as has been shown by Montgomery, Sutton and others, the spermatogonial and oögonial chromosomes may be paired two by two in respect to their size relations. In *Galgulus*, the chromosomes are too nearly equal in size to be readily paired, and the differential chromosomes are indistinguishable from the others.

Since in *Galgulus* there are two classes of spermatozoa; since the spermatogonial number is thirty-five and the oögonial thirty-eight, we have another support to add to the view that the two classes of spermatozoa are respectively male and female producing. In a recent paper, Correns has demonstrated by experiment, that in some plants there are two kinds of male germ cells produced in equal numbers, and that these two kinds are male and female determinants.

Zoölogical Laboratory,
Columbia University,
February 21, 1908.

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OCCURRENCE OF THE FRESH-WATER MEDUSA, LIMNOCODIUM, IN THE UNITED STATES.[1]

CHAS. W. HARGITT.

In a preliminary note to *Science*, which appeared in the issue of November 8 (1907), attention was directed to the occurrence of *Limnocodium* in this country, and some brief account given of this circumstances and conditions of its appearance. As indicated in that report a few living specimens came into the possession of the writer through the courtesy of the United States Bureau of Fisheries, and were kept alive and under observation for a period of some ten days. Since it was confidently anticipated that a further supply of specimens would be received very soon no particular care was taken to prolong the life of the first supply. A few were used for some simple experiments, to be described in a later section, and some preserved for histological purposes. It is with extreme regret that I have to record the utter failure of attempts to secure a further supply of living specimens, though from no particular blame of those concerned. At least one lot was forwarded to the writer at Syracuse, and left Washington in good shape, but from some cause not clear all were dead when received, having perished in transit. Other supplies were immediately sought, but within a few days the very disappointing advice was received from the director of the aquarium that the medusæ had suddenly disappeared. Quoting his own words: "When I went to the tank where they have been so plentiful for the past eight weeks there was not one to be seen, and apparently they have disappeared as mysteriously as they came. Possibly on account of the cold weather they may have gone to the bottom of the tank, among the pipes where I cannot get them, but if I discover them again I will advise you at once." This letter bore date of September 27. In a later letter, December 3, I was advised that no specimens had appeared during the interval of two months, and from the history of these medusæ in Europe this is only what might have been anticipated. Therefore, with the first chapter

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

of the present case thus closed, the details, so far as known, may properly be formulated and made available.

Locality.

The medusæ were discovered in the aquarium of Mr. W. B. Shaw, a florist, who for many years has cultivated various aquatic plants, among them several species of tropical, or subtropical water lilies, *Nymphæa zanzibarensis*, *Cabomba* (*Caroliniana?*), and a species of *Ludwigia*. But, *all these were reared from seeds*, no grown plants having been at any time imported or introduced into the tanks. And at no time has the *Victoria regia* been grown here, a fact of no small interest in relation to earlier suggestions as to the problem of the transportation of the medusæ from tropical waters.

The hot-house contained in all six tanks, each three feet wide, three feet deep, and about twelve feet long. These were all stocked in the same way, and with about the same sorts of plants, and a species of Paradise fish. The aquaria are of the balanced sort, and have been in use for some six or eight years without material change of water or organisms. The aquaria are used chiefly during the winter by Mr. Shaw including late fall and spring, for the purpose of carrying over certain of the more delicate tropical plants from season to season, and stand idle during summer, water being added to replace the loss due to evaporation.

Of these six tanks, alike in construction, water supply, and other features, medusæ appeared in *one only*, and that about mid-summer, flourishing during the hot weather and promptly disappearing with the approach of autumn cold. When first discovered they were in considerable numbers and of various sizes "some as small as a pin's head and some one fourth inch in diameter." The largest specimen which came to my hands measured about 9 mm. in diameter by 4 mm. in height of bell.

Hoping to obtain some clue as to the hydroid stage I obtained through the courtesy of the Bureau of Fisheries a collection of various plants growing in the tank, with scrapings of algal slime and other debris from the sides and bottom of the tank. This was promptly and carefully examined on arrival, and frequently

during succeeding days, but no trace of a hydroid was found. The material was kept in my laboratory, which has a fairly constant temperature, for several weeks, indeed months, and repeated examinations made from time to time, but no trace of any hydroid form appeared, though species of Protozoa and rotifers continued to live and thrive quite normally. Such are the facts as to locality and conditions under which the medusæ were found. Their similarity to those concerned in the appearance of the same medusa in London many years ago is quite striking. Its occurrence later at several places on the continent has been recorded by several observers. The most recent is that of its appearance at the botanical garden at Munich in the basin containing the giant water lily, *Victoria regia*. Boecker's account, which appeared in the *Biologisches Centralblatt* (Bd. XXV., p. 605, 1905), agrees in most respects with the earlier records, and with the foregoing as well. He regards the Munich species as probably identical with *Limnocodium sowerbii*, the occurrence of which in the Victoria tanks of Regents Park, London, had been variously described by Lankester (*Quar. Jour. Mic. Sci.*, Vol. XX., 1880), Allman (*Jour. Linn. Soc.*, Vol. XV., 1881), and Gunther (*Quar. Jour. Mic. Sci.*, Vol. XXXV.). Some further notice will be taken of Boecker's records in a later connection.

General Features and Relations.

The Washington species agree in most respects quite closely with the original descriptions and figures of *Limnocodium sowerbii*, of Allman and Lankester. As compared with the verbal description of Boecker (*op. cit.*), there are some slight differences. For example, he says that the Munich species are characterized by a rather high-arched bell. In my species the bell is rather low, often disc-like. Figs. 1, 2, 3, 4, drawn from life in different aspects, will make this point more evident and striking. However, this difference I do not consider of very great importance.

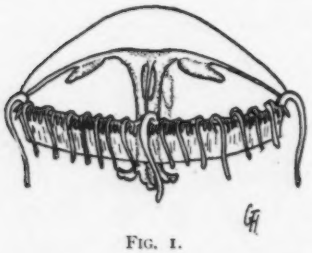

Fig. 1.

Several things might conspire to emphasize or exaggerate it. As is well known, age has much to do with the shape of hydrome-

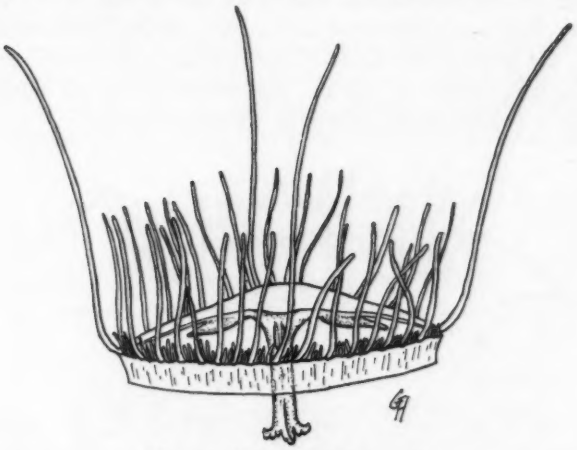

FIG. 2. Medusa, floating aspect.

dusæ, young specimens often being hemispherical, while at maturity they may become quite disc-like. Again, there are often marked individual differences which have to be considered in estimating the mean shape of the species. Further, preserved specimens are almost always more or less contracted, and hence may seem to be much more highly arched than is the case in life.

It should be observed in this connection that the specimens shown in the figures present very different aspects. Fig. 1 represents the medusa in an average swimming attitude, the tentacles more or less contracted, the manubrium somewhat contracted, and the bell rather higher than shown in Fig. 2, which represents the creature in the floating attitude, body and organs generally

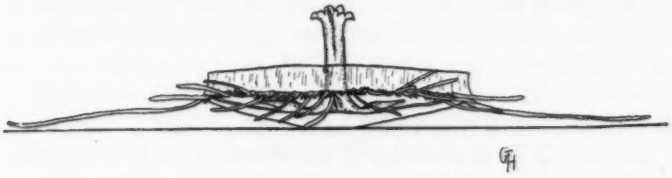

FIG. 3. Medusa, resting normally on bottom of aquarium.

extended, and more or less passive. In Fig. 3 the specimen is represented in a rather characteristic state of repose often observed, resting on the exumbrellar surface on the bottom of the aquarium. Here again, the creature is in a condition of general relaxation, the manubrium extended and in position to take such prey as may come within its reach. The tentacles also are much extended, a condition quite common when in this pose.

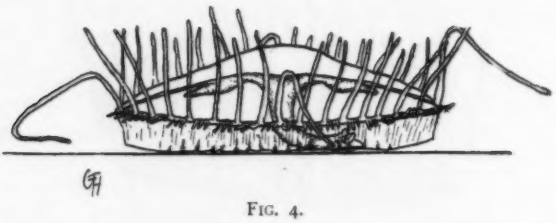

FIG. 4.

In Fig. 4 is shown a less familiar attitude of the creature. Though not especially rare, the position seems to indicate a condition of fatigue, though the tentacles are more or less erect. A comparison of the several figures, representing as closely as possible the living aspects of the creature, will show the general relation of tentacles, velum, manubrium, shape of bell, etc., to the state of activity or repose, as the case might be.

TENTACLES.

There are four perradial tentacles, conspicuous as to size and length, as shown in the several figures. In certain cases it was possible to distinguish apparently, a series of four interradial tentacles though they were not conspicuously differentiated as to size or length. The tentacles are quite numerous, especially in older specimens, and arranged in several series, younger ones arising from the lower, or proximal portion of the margin. While the aspect exhibited by these organs in the figures might seem to indicate more or less rigidity of structure, this did not appear to be the case in the living specimens. They were quite flexible and highly contractile.

In describing the tentacles of the Munich specimens Boecker (*op. cit.*) was unable to confirm the account given by Günther as

to the insertion of the base into the gelatinous margin of the bell, believing them to be quite free. He does not state whether he had studied this feature in sections, or from simply observing the living specimens.

On this point my specimens show considerable differences. In the living condition, the larger tentacles, while capable of great freedom of movement, are yet inserted in notch-like depressions in the margin, as shown in Fig. 5. As studied in sections they

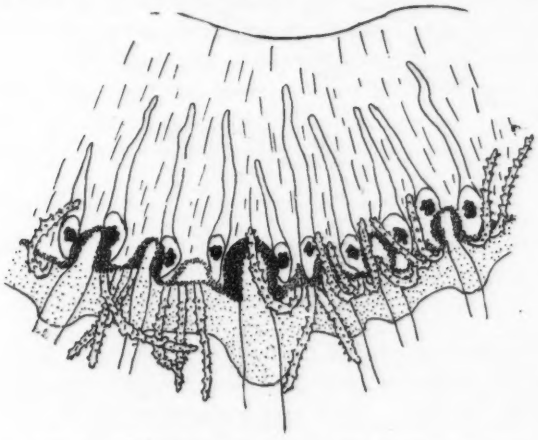

FIG. 5. Portion of margin enlarged, showing tentacles, velar canals and marginal bodies.

show something of the condition figured by Günther, though very much less marked, the degree of adnation being much less than in his description. Furthermore, I should not consider the degree of fusion as having any very evident relation to the aspects of the tentacles exhibited by the living medusæ. On the contrary, the varying aspects of these organs under differing conditions of activity or repose are such as to strongly suggest a very different conclusion and interpretation.

A further tentacular feature remains to be considered, namely, that of structure. Both Allman and Lankester described the tentacles as solid, though differing more or less from that found in other medusæ with solid tentacles. Günther has later shown that these conclusions were erroneous, and attributes

the error as possibly due to an examination of very young tentacles, or to tentacles in a state of high contraction.

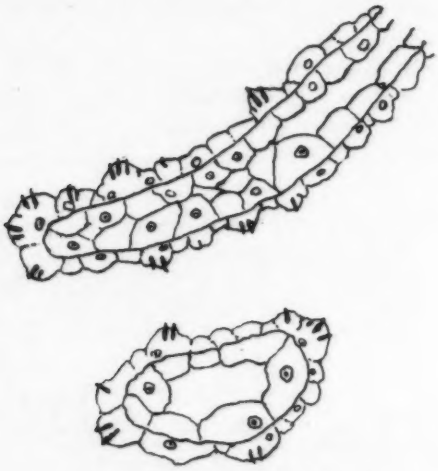

FIG. 6.

My own sections confirm the results of Günther. In sections of very small tentacles it is quite difficult to distinguish the lumen of the tentacular axis. But in larger specimens this is quite easily demonstrated, and it seems rather strange that two such capable observers should have failed to recognize this feature. Fig. 6 shows camera sketches of both cross, and long sections of tentacles, and Fig. 7 is a photograph of the terminal portion of a tentacle made under the microscope.

FIG. 7. Tentacle of Limnocodium.

In this connection I desire also to point out that in none of my specimens have I found the very marked papillated aspect of the tentacles as represented by both Lankester and Günther (*op. cit.*), and later by Browne (*Quar. Jour. Mic. Sci.*, Vol. 50, Fig. 3, pl. 37). These papillæ are specialized organs bearing nemato-

cysts, according to these observers. But I have failed to find any well defined cases of such highly specialized nematophoric structures. The figures, both from the drawings and the photograph alike, confirm this statement. I cannot resist the impression that the figures of the former must have been drawn from greatly distorted specimens due in part perhaps to mode of preservation, or that they may have been made from very young tentacles in which the nematocysts are relatively more conspicuous. A comparison of various of my specimens fails to reveal any essential differences, even where various modes of preservation were employed. An examination of the specimen represented in Fig. 7 will show fairly well the general distribution of the nematocysts, and also the tendency to an annular disposition similar to that common to other hydromedusæ.

Marginal Bodies and Velar Canals.

Concerning these organs I have made no attempt to critically investigate anew their structure or development, beyond a more or less careful review of the work of Lankester, and the later work of Günther (*op. cit.*). So far as I have gone my observations confirm that of the previous investigators. Fig. 5 is a camera sketch of a portion of the margin and velum showing the general aspects of the several organs, tentacles, marginal bodies and capsules, and velar canals. But while confirming the facts described by Lankester as to the structure and relations of the marginal bodies, I have found no convincing evidence of his rather dogmatic assertion that "The refringent body is nothing more nor less than a modified tentacle." This may have sufficed its author at the time it was made, and was quite in harmony with then current views concerning these organs in general. But the time has long since passed when the mere matter of similarity of structures, real or imaginary, is any sufficient warrant for the vaulting conclusion involved in the above quotation. In another connection (*Jour. Exp. Zoöl.*, Vol. I., p. 86), I have taken occasion to question the long current assumption that in Scyphozoa the rhopalia are metamorphosed tentacles. Later observations have only served to deepen the conviction, and I believe adequate investigation will confirm the view that these organs are not, in

any strict morphogenic sense, directly derived from tentacles, particularly such bodies as those here under consideration.

Reproduction.

There is nothing to add to the former announcement as to this feature. Only male specimens have been found. These were in various stages of maturity, some quite young, others sexually mature, with gonads bursting with ripe spermatozoa. In the absence of additional facts there is no particular call for speculations as to the significance of this singular sexual phenomenon.

Affinities of the Medusæ.

Concerning the question of the specific or generic relations I find little occasion for difference of opinion or doubt. That our specimens belong to the genus *Limnocodium* seems thoroughly clear and certain. That it is also specifically identical with the European species, *L. sowerbii* Lankester, seems almost equally certain. I have pointed out certain features of difference, such as the shape of the bell as compared with Boecker's description, and peculiarities of tentacular structure, rather sharply different from those described by the earlier observers. Yet neither of these, nor both combined are such as to warrant the probability of a distinct species. Hence the suggestion made in my preliminary report may be here reiterated, namely, that the American species is almost certainly *Limnocodium sowerbii*.

The following characters may be regarded as fairly diagnostic of the species: Bell rather low, two or three times as broad as high, discoid in shape, especially when floating freely as shown in Fig. 2. Size from 5 to 9 mm. in diameter, by about 4 mm. in height, or less in average specimens; radial canals four, rather capacious, extending from stomach to marginal canal, which is triangular in shape and large; gonads four, suspended from the radial canals about midway between the stomach and margin, pouch-like in shape with the smaller or distal end free, as shown in the figures, pale greenish in color; manubrium long, and with four more or less crenulated lip-like lobes which are of greenish tint, and extending usually considerably beyond the velum; tentacles very numerous, the four perradial ones being conspicuously

long and usually extended upward over the bell in graceful curves; other tentacles in several cycles, the newer arising along the inner edge of the margin; the older ones set in notch-like scallops about the margin, as shown in Fig. 5. The general color of the bell translucent, or of faintly bluish tint by reflected light. Bases of tentacles surrounded by a dull brownish, but inconspicuous pigment.

Experimental.

The extensive experimental work which has been done upon medusæ within recent years, and the various results which have been obtained by different experimenters, at once suggested the desirability of repeating certain phases of it upon a fresh-water medusa. Accordingly I devised a series of experiments to test its regenerative capacity along lines similar to those made by the writer on *Gonionemus*, *Rhizostoma* and others; to test its behavior in response to various stimuli — photic, chemical, mechanical, tactile, etc. Having received less than a dozen living specimens in the first lot it was of course out of the question to set about any systematic and extended experimentation at that time, fully anticipating ample material later for the work planned. As already intimated in the introduction, these plans were doomed to miscarry, owing to the utter failure in securing the necessary material for their realization, and but for a single series of very simple experiments this feature of the work would have been wholly lacking. The experiments alluded to had to do wholly with the reaction of the medusæ to distilled water.

The numerous experiments to determine the rôle of certain salts in relation to the rhythmic movements of medusæ are too well known to call for extended review. On the one hand it is claimed that the presence of certain ions (Na), is the all-important factor in arousing and sustaining the movements, while the presence of certain other ions (Ca, K), have an inhibitory effect upon them. "Na ions start or increase the rate of spontaneous rhythmical contractions; Ca ions diminish the rate or inhibit such contractions altogether."

On the other hand it has been claimed that Mg ions are the magic factor which causes the organism to perform its rhythmic

play. Still others have contended that various species react very differently to a given stimulus, and that an element which may prove a stimulus in one case may prove inhibitory or even toxic in another case. It is quite certain, therefore, that the last word has not yet been said concerning this perplexing problem. And so far from the rather dogmatic declaration that "it is only the presence of Ca ions in the blood which prevents the muscles of our skeleton from beating rhythmically in our body" we may better assume an attitude of open skepticism, or at least suspend judgment long enough to perform a few more experiments! In the lack of material for such experimentation in the present case it occurred to me to try in a very simple fashion the effects of perfectly pure, that is distilled, water on the medusæ. In the absence of the ions of the aforementioned elements, Na, Ca, K, etc., in such water should we get any of the results usually attributed to them? The problem seemed simple enough, but not too simple to be unworthy of a trial. Accordingly the following experiments were made:

1. Specimens were transferred from the water in which they had been sent from the aquarium to that of the ordinary tap-water of the laboratory in order to see whether any appreciable effect would follow. But none could be observed; hence they were thereafter freely transferred to such water as circumstances seemed to suggest from day to day. Later tests of this water showed it to be so free from any of the salts in question as to be indistinguishable by the ordinary chemical tests.

2. A single specimen was next transferred to a jar of distilled water. It moved rhythmically for thirteen beats, then paused. Then followed six beats succeeded by another pause of longer extent during which it floated downward sinking to the bottom. Here there followed five beats and a pause of five minutes. At this time it was transferred back to normal water with the result of prompt resumption of rhythmic activity, but of a more rapid rate, nearly or quite twice that before the experiment was begun. This continued for nearly ten minutes, when the rhythm gradually returned to the normal rate.

3. Another specimen tested in the same way gave almost identically the same reactions.

4. The next specimen, somewhat larger than either of the former, was transferred to distilled water, and pulsed rhythmically twenty-three times, then paused as had the former. Then followed thirteen pulsations with another pause of similar extent, during which it gradually sank to the bottom where it remained motionless for three minutes. It then resumed its rhythmic swimming and continued without further pause for ten minutes when it was returned to the normal water.

5. After about twenty-five minutes all three specimens were transferred at once to a fresh jar of distilled water. In this case the reactions were similar throughout, though with certain individual differences such as one might expect. All swam rhythmically for a half minute, and then paused as in the previous cases. The rhythm was resumed and after a brief time two of the specimens paused again, and as before sank to the bottom of the jar, the third continued swimming. After a short rest those on the bottom resumed their swimming, and all continued for nearly half an hour, just as in normal water. They were left in that condition for twenty minutes longer, when two were found to have sunk to the bottom once more and seemed to show sign of discomfort, such as the contraction of the tentacles, drawing in of the margin of the bell, etc. The third while still swimming, was evidently showing unusual signs of fatigue, the movements being more or less feeble and uncertain. All were at once transferred to normal water. Two soon showed signs of recovery, the third however, continued in a state of collapse and failed to further recover. All had evidently been injured by the long period in the distilled water, and one fatally, since it was later found dead without indications of any recovery in the normal water.

Such in briefest outline are the facts resulting from the few experiments made. It should be stated that they were variously repeated, and with such degree of constancy in behavior as to suggest their perfectly trustworthy nature. Still other experiments of the same sort would have been made, but for the confident anticipation of a large supply of fresh specimens.

In reviewing the literature of this medusa it has been gratifying to find that some experimental work had long ago been done

upon it. In 1880 Romanes (*Nature*, June 24; also "Jelly fish, Star fish, etc.," 1885), whose experiments on various medusæ are well known classics, took occasion to repeat certain of them on *Limnocodium*, chiefly in relation to light, temperature, tactile localization, and reactions to sea water. In most respects he found the reactions of *Limnocodium* comparable in almost every respect with those performed on marine species. His experiments in transferring the specimens to sea water was to test their tolerance to a medium and environment to which their supposed ancestors must have long been familiar, are interestingly the very opposite of those above described. He had previously proved that marine species were unable to endure a transfer for more than ten minutes to fresh water, seldom for more than five, and then with evident signs of more or less severe injury. In the reverse tests with *Limnocodium* he found that no reaction was apparent for about fifteen seconds, when a more or less sudden collapse resulted, with tetanus-like spasms of increasing intensity till all sensory reaction ceases, and after a short time death ensues. An exposure to sea water for even one minute may end fatally, even if the creature be promptly returned to fresh water. Similar results followed an immersion in dilute sea water though with less promptness. In very reduced sea-water ($\frac{1}{10}$), spontaneous activity may continue for some time, but with gradual decline till but slight response is given to stimuli.

In conclusion he states "It will be seen from this account that the fresh-water medusa is even more intolerant of sea water than are the marine species of fresh water. It would appear that a much less profound physiological change would be required to transmute a sea water jelly fish into a jelly fish adapted to inhabit brine than would be required to enable it to inhabit fresh water. Yet the latter is the direction in which the modification has taken place, and taken place so completely that the sea water is now more poisonous to the modified species than is fresh water to the unmodified. There can be no doubt that the modification was gradual — probably brought about by the ancestors of the fresh-water medusa penetrating higher and higher through the brackish waters of estuaries into the fresh waters of rivers, and it would, I think, be hard to point to a more remarkable case of

profound physiological modification in adaptation to changed conditions of life."

In the summer of 1905, as already pointed out, this medusa appeared in considerable numbers in the aquaria of the Botanical Garden of Munich. In a recent paper (*Zeits. f. allgemeine Physiol.*, Bd. VII.), Maas has recorded a series of experiments made to test the reactions of the medusæ to various stimuli, chiefly chemical and mechanical, with brief references also to the influence of temperature. Experiments made with physiological salt solution confirmed in a general way those of Romanes already considered. For example, specimens when first placed in the solution showed quickened, spasmodic and heterochronous contractions, followed later by collapse and finally by death. If left in the solution for only ten minutes and then returned to the normal water the irregularity gradually disappears and the pulsations become synchronous and normal. That these reactions were not due to any difference in temperature he proves by control experiments made on various specimens at the same temperatures in fresh water, and even in water at very much lower temperatures.

He also tested the reactions of emarginated specimens in the salt solutions and found the same spasmodic contraction as observed in the uninjured specimens. A brief summary of the comparative effects of KCl and NaCl is given, from which it would appear that the former tends to inhibit reactions, while the latter serves as an active stimulus, though of a very irregular or heterochonous character.

Maas also points out the somewhat conflicting results of Loeb's experiments on *Gonionemus* and *Polyorchis*, those of Bethe on *Olindias*, and Herbst on *Obelia*; and concludes that among marine medusæ there must be essential differences in their chemico-physical relations.

In the transfer of a delicate creature, such as medusæ, from a medium like sea water to that of fresh water it might naturally be expected that reactions more or less remarkable should occur. But what shall be said of similar reactions which follow the transfer from a fresh-water medium, where if any soluble salts be present it must be in homœopathic doses of high attenuation,

to distilled water devoid of any trace of these elements? Surely it can hardly be claimed here that continued rhythmic action, or its inhibition, is attributable in any specific way to the ions of *this*, *that*, or *any* sort! Possibly those who would vindicate the ion hypothesis at all hazards might assume, as a desperate measure, that certain infinitesimal quantities of these elements may have been carried over in the tissues of the medusæ in their transfer from the normal to distilled water! But the assumption is so wholly gratuitous as to preclude serious consideration.

The writer has no theoretical views to promote or defend. But face to face with the facts he assumes, what is the right of every investigator, namely, freedom to discard any hypothesis which fails to account for his facts, and in so doing the further duty of exposing its futility. A comparison of the experiments heretofore set forth, and those to which direct reference has been made, together with others of similar nature and involving similar methods, shows such degree of confusion, not to say absolute contradiction, in the results obtained, as to suggest pause and serious reflection before any hasty leap be made in formulating conclusions. There is an old, but reputable, saying though involving something akin to paradox: "If the light that is thee be darkness, how great is that darkness." In the light of existing darkness concerning the problem under consideration might not some modium of scientific modesty and hesitation well replace something of the arrogance and dogmatism which have bulked so large in recent literature of these problems?

SYRACUSE UNIVERSITY,
February 25, 1908.

AN UNUSUAL GRAAFIAN FOLLICLE.

ELLISON A. SMYTH, JR.

In the fall of 1906 I became possessed of a young setter pup, with an interesting family history: her mother a rather small Gordon setter, had given birth to her first litter, to which my pup belonged, in June, 1906, which litter consisted of fourteen pups, four dogs and ten sluts. Two died when three or four months old, and the rest were successfully raised. The mother had seven tits in lactation.

For reasons of convenience I had this pup spaed in March, 1907, she being then ten months old. The ovaries I killed at

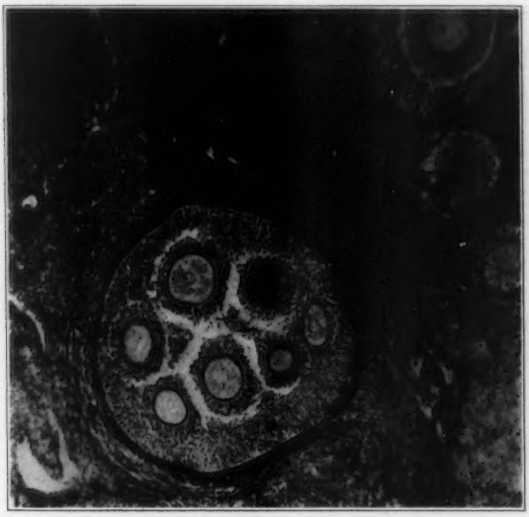

FIG. 1.

once in Flemming's solution and sectioned for class use. They showed a number of ripe follicles, not a few containing double and triple ova, though not all the extra ova were normal. Several serial sections on one slide, however, showed a large follicle

about ripe with two perfect ova at opposite poles; in each of these ova the nucleus was normal and the chromatin elements and network and also nucleolus perfect and beautifully distinct. Unfortunately I partially destroyed this slide through a vexatious accident. I obtained, however, another pair of ovaries from another pup, and on sectioning found many follicles with two, a number with three, a few with four, and one follicle with seven ova in clear view. As far as I can ascertain this is the greatest number of ova recorded from any mammalian follicle. The case is especially interesting on account of the family history. The mother setter has never had another litter. One of her daughters littered in July, 1907, having nine pups, three dogs and six sluts, with eight tits in lactation.

These facts are interesting in their suggestiveness along heredity lines, and also as to the relation between multiple ova in follicles, and large number of offspring.

The micro-photograph is of this follicle of seven ova, × 90.

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